

USSN 09/448,042

Amended Claims in Mark-Up Format

1. (Thrice Amended) A method for determining the presence of a target nucleotide, the method comprising the steps of:

(a) exposing a biological sample selected from the group consisting of blood, stool and urine to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;

(b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-attached acceptor molecule comprising a fluorophore or a fluorescent dye, wherein, upon incorporation of said dideoxy nucleotide into a double-stranded nucleic acid product resulting from the primer extension reaction, said acceptor molecule is proximate to said donor molecule such that said acceptor molecule is [being capable of being] activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal without self-quenching [when said dideoxy nucleotide is incorporated into a product resulting from the primer extension reaction];

(c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the double-stranded nucleic acid [primer extension] product; and

(d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the double-stranded nucleic acid [primer extension] product.

25. (Amended) A method for determining the presence of a target nucleotide, the method comprising the steps of:

- (a) exposing a biological sample comprising a bodily fluid to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;
- (b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-attached acceptor molecule comprising a fluorophore or a fluorescent dye, wherein, upon incorporation of said dideoxy nucleotide into a double-stranded nucleic acid product resulting from the primer extension reaction, said acceptor molecule is proximate to said donor molecule such that said acceptor molecule is [being capable of being] activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal without self-quenching [when said dideoxy nucleotide is incorporated into a product resulting from the primer extension reaction];
- (c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the [primer extension] double-stranded nucleic acid product; and
- (d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the double-stranded nucleic acid [primer extension] product.

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Clean Copy of Pending Claims

1. (Thrice Amended) A method for determining the presence of a target nucleotide, the method comprising the steps of:

(a) exposing a biological sample selected from the group consisting of blood, stool and urine to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;

(b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-attached acceptor molecule comprising a fluorophore or a fluorescent dye, wherein, upon incorporation of said dideoxy nucleotide into a double-stranded nucleic acid product resulting from the primer extension reaction, said acceptor molecule is proximate to said donor molecule such that said acceptor molecule is activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal without self-quenching;

(c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the double-stranded nucleic acid product; and

(d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the double-stranded nucleic acid product.

4. The method of claim 1, wherein said extension reaction is performed in the presence of at least two different dideoxy nucleotides, each comprising a different acceptor molecule that produces a distinct fluorescent signal upon activation.
10. (Twice Amended) The method of claim 1, wherein said fluorescent dye is selected from the group consisting of 6-carboxyfluorescein (FAM), 6-carboxy-X-rhodamine (REG), N₁, N₁ N¹, N¹-tetramethyl-6-carboxyrhodamine (TAMARA), 6-carboxy-X-rhodamine (ROX), fluorescein, Cy5® and LightCycler-Red 640.
11. The method of claim 1 wherein said donor molecule comprises 6-carboxyfluorescein (FAM).
12. The method of claim 11 wherein said acceptor molecule comprises, 6-carboxy-X-rhodamine (ROX).
15. The method of claim 1 wherein said dideoxy nucleotide is a 2'3'-dideoxy nucleotide triphosphate selected from the group consisting of ddATP, ddCTP, ddGTP, ddTTP and ddUTP.
18. The method of claim 1, wherein said target nucleotide is present as a result of a nucleic acid mutation.
19. (Amended) The method of claim 18, wherein said mutation occurs in a gene selected from the group consisting of ras oncogenes, p53, dcc, apc, mcc and β -catenin.
20. The method of claim 4, wherein said target nucleotide is present at a single nucleotide polymorphic locus.
25. (Amended) A method for determining the presence of a target nucleotide, the method comprising the steps of:

(a) exposing a biological sample comprising a bodily fluid to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;

(b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-attached acceptor molecule comprising a fluorophore or a fluorescent dye, wherein, upon incorporation of said dideoxy nucleotide into a double-stranded nucleic acid product resulting from the primer extension reaction, said acceptor molecule is proximate to said donor molecule such that said acceptor molecule activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal without self-quenching;

(c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the double-stranded nucleic acid product; and

(d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the double-stranded nucleic acid product.

26. The method of claim 25, wherein said bodily fluid is selected from the group consisting of pus, semen, sputum, saliva, cerebrospinal fluid, biopsy tissue and lymph.

27. The method of claims 1 or 25, wherein said biological sample is obtained from a pooled patient population.